Influence of some oenological practices on the aromatic and sensorial characteristics of white Verdejo wines

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Abstract Changes in the aromatic composition as well as sensory characteristics in Verdejo white wines were analysed based on two factors: the winemaking methodology and the storing time of wine in bottles. The volatile components were determined by GLC-MS, and the sensory profile was designed and assessed according to the ISO 11035 standard. The results showed that when wines were made in oak barrels, either completely or partially, which means the wines were in contact with the lees, the levels of 1-octanol, ethyl heptanoate and ethyl decanoate were significantly affected ($P < 0.05$); the softness sensation was also influenced ($P < 0.05$). However, the amount of time the wines were stored in bottles significantly affected ($P < 0.05$) the levels of 1-hexanol, ethyl heptanoate, ethyl octanoate, ethyl decanoate, hexyl acetate, isoamyl acetate and isoamyl lactate and also an odour note (tropical fruit). The compounds with higher OAV values belong to the groups of esters and fatty acids. For these reasons, the composition and the quality of the aroma of Verdejo white wines appear to be significantly affected both by use of oak barrels in winemaking and the time the wines are stored in bottles.

Keywords Aroma components, bottle ageing, lees, oak barrel, olfactive active value, sensory analysis, Verdejo wines.

Introduction

As in many other types of food, aromas are an essential parameter of quality in wines, particularly in white wines. The aromas may be of a varietal, prefermentative, fermentative or postfermentative origin. Most wines made in bulk need special attention to be given to the aroma quality originating in the alcoholic fermentation, which is significantly affected by the grape variety, the stock yeast used, the macromolecules of the initial must, the fermentation temperature and the oenological practices (Valero *et al.*, 2002; Flanzy, 2003; Torija *et al.*, 2003; Bueno *et al.*, 2006). Furthermore, white winemaking may also highlight the varietal characteristics, and there is another group of white wines in which the postfermentative aromas are enhanced by means of different oenological practices such as skins maceration and ageing on lees. The ageing of white wines on lees enables us to modify the aromatic and sensorial characteristics of the wines (Feuillat & Charpentier, 1982; Chung, 1986; Feuillat *et al.*, 1989; Pueyo *et al.*, 2000; Fornairon-Bonnefond *et al.*, 2002; Comuzzo *et al.*, 2006). This may be due to the release of the yeast components, the fixation of the aroma components because of the insoluble fraction of the lees, or the various reactions of formation or hydrolysis of volatile components. This wide range of possibilities makes it difficult to assess the effect of the lees on the aroma of the wines (Perez-Serradilla & Luque de Castro, 2008). The molecules released originate either inside the cells (peptides, amino acids, nucleotides, etc.) or in the cell walls (glucans and mannoproteins), as it has been shown by Ferrari & Feuillat (1988), Guilloux-Benatier *et al.* (1995) and Alexandre & Guilloux-Benatier (2006). The volatility of some wine aroma components may be affected by their interaction with constituents of the soluble fraction of the lees, the interactions with the mannoproteins, those being the most widely studied (Comuzzo *et al.*, 2006; Chalier

et al., 2007). Similarly, the insoluble fraction of lees may also fix aroma components, such as 4-ethylphenol, ethyl acetate or mid-chain fatty acids: this fact results in the decrease in their concentration in the medium (Chassagne *et al.*, 2005; Comuzzo *et al.*, 2006; Pradelles *et al.*, 2009). The total content of nitrogen and some amino acids, which are precursors of volatile components such as mid-chain alcohols and lactones, increase while wine is in contact with the lees (Bueno *et al.*, 2006). In addition, the reactions of esterification and hydrolysis may be strongly affected by the activity of the esterases released by the lees after the alcoholic fermentation (Mauricio *et al.*, 1993; Zoecklein *et al.*, 1997). If the ageing on lees takes place in oak barrels, the content of certain volatile compounds given off by the wood may increase: these include lactones, volatile phenols, furanic derivates and phenolic aldehydes, although wines aged on lees in oak barrels have a less woody character than those in which lees have been prematurely removed (Jiménez-Moreno & Ancín-Azpilicueta, 2007). In addition, the aromas generated during the alcoholic fermentation may undergo different processes inside the oak barrel, such as their absorption by wood, which decreases their content (Ramirez-Ramirez *et al.*, 2004; Jarauta *et al.*, 2005), or their protection by the lees, which limits their oxidation (Chatonnet *et al.*, 1992). The release of a significant colloidal load in wines during yeast autolysis, mainly owing to mannoproteins and glucans, which means an increase in the structure of wine in the mouth, that enhances the sensation of softness and density in sensory analysis (Fornairon-Bonnefond *et al.*, 2002).

During the ageing of wine in bottles, several chemical changes in the volatile composition take place. This is owing to a series of reactions that depend on the composition of wine (concentration of ethanol, pH), the time the wine is stored in the bottle and the storing temperature (Marais, 1978; Rapp & Mandery, 1986; Flanzy, 2003). Most ethylic esters and acetates hydrolyse, while the concentration of the esters of other organic acids increases and the terpene compounds disappear or change into other terpene compounds with higher levels of oxidation.

Verdejo is the main white variety of the *Apellation d'Origine Contrôlée* Rueda (Castilla y León, Spain), a total of 10 580 hectares having been grown in 2010. These are the quality white wines most commonly consumed in Spain, and they are becoming more and more popular. However, although some studies on their aromatic composition have been carried out (Rodríguez-Nogales *et al.*, 2009; Sánchez-Palomo *et al.*, 2010), there is little information on the effect on the aroma composition of certain oenological practices, as industrial winemaking in stainless steel tanks, the making of wine in oak barrels, and the storing of wine in bottles. Thus, the purpose of the research was to study some technological factors that may affect the composition and the aroma quality of white Verdejo wines, including the storing in bottles before consumption.

Materials and methods

Reagents and standards

All reagents used were analytical graded. Analytical standards for GLC and sensory analysis were obtained from Sigma-Aldrich Química (Tres Cantos, Spain), except Mannoplus® and mid-toast American oak wood chips provided by Agrovin (Alcázar de San Juan, Spain).

Grapes and winemaking

Verdejo grapes were collected in a 18-year-old vineyard located in the area of *Appellation d'Origine Contrôlé* Rueda (province of Valladolid, Spain), at about 750 m a.s.l. Plants, grafted on 110 Richter and trained on double Guyot system, are arranged N–S, vine spacing being 2.80 × 1.50 m. Irrigation was carried out using a drip irrigation system, and production was about 8500 kg ha^{-1}. Harvest took place around the end of September 2007, and grapes presented an optimum sanitary state. Grapes (3000 kg) were hand-harvested, placed in 30-kg plastic boxes and transported to the winery. Winemaking was carried out in Vinos Blancos de Castilla S.A. cellar (Rueda, Valladolid, Spain). Grapes were crushed and de-stemmed, and they were later treated with sulphur dioxide (60 mg L^{-1}) to avoid must oxidation. Skin maceration took place in a pneumatic press during 8 h at 10 °C under N_2. Then, crushed grapes were pressed for 75 min, maximum pressure being 2 bar. Must recovery was 64%. Must was settled in a 2,000-L stainless steel tank for 24 h at 14 °C, limpidity was 140 NTU after settling. Afterwards, must was poured into four 250-L stainless steel fermentation tanks and two 225-L middle-toast American oak barrels (Magreñán, Logroño, Spain). Tanks and casks were located in a room at 17 °C; 0.30 g L^{-1} Uvaferm CS2 yeast (Lallemand España, Madrid, Spain) was added, and temperature and must density were controlled twice a day. The must-wine remained in four containers (two tanks and two barrels) till the end of alcoholic fermentation. The must-wine from the other two tanks was transferred to other two oak casks when density was 1.0300 and remained there till the end of alcoholic fermentation.

When the alcoholic fermentations finished, sulphur dioxide was adjusted to 30 mg L^{-1}, to avoid the development of malolactic fermentation. Then, wines made in stainless steel tanks were transferred to other tanks in the same room, remaining without lees for 6 months, but wines made in oak casks remained in the

same casks in contact with their lees, for 6 months, the lees being stirred once a week during 5 min, using a mechanical stirrer developed by Vinos Blancos de Castilla winery. Then, wines were clarified with sodium bentonite (1.00 g L^{-1} for A wine, 0.80 g L^{-1} for AB wine and 0.65 g L^{-1} for B wine) and potassium caseinate (100 mg L^{-1}), taking into account the results obtained after Giacomini test (Giacomini *et al.*, 1995), filtered using a filter aid (white diatomeaceous earth), transferred to a chamber at −3 °C for 13 days to achieve tartrate stabilisation and finally filtered with rosé diatomeaceous earth. The content of sulphur dioxide was corrected, and wines were filtered through a 0.80-μm membrane before bottling in 0.75-L bottles, using class A natural cork stoppers. Bottles remained in a subterranean cave, at 14 °C and 80% relative humidity.

Summarising, six different wines were obtained; the following codes being used for their identification: A, winemaking in stainless steel tanks; AB, winemaking in stainless steel tanks and in oak casks; B, winemaking in oak casks; 1, bottle ageing for 1 month; 7, bottle ageing for 7 months. Thus, A-1 wine was made in a stainless steel tank and aged in a glass bottle for 1 month.

General analysis of musts and wines

Must limpidity was determined using a HI93703C portable turbidimeter (Hanna Instruments, Eibar, Spain). General analysis of wines was carried out 30 days after bottling. Density, alcoholic degree, dry extract, reducing sugars, total acidity, volatile acidity, pH, free sulphur dioxide, total sulphur dioxide and tartaric, malic and citric acids were determined by OIV methods (OIV, 2000).

Analysis of volatile compounds in wines

Volatile compounds were determined by GC-MS, 1 and 7 months after bottling. Extraction of volatile compounds was carried out by the trap headspace technique, using a Turbo matrix 40 Trap (Perkin Elmer, Waltham, MA, USA), using for purge per trap cycles, the vial being pressurised for 1 min and charged for 1.5 min. The following temperatures were used: needle, 110 °C; oven, 85 °C; transfer line, 140 °C; trap, 45 °C (low) and 290 °C (high). Pressures were as follows: vial, 30 psi; in column, 30 psi; and desorption, 30 psi. Times stipulated for the different processes that take place in the headspace were as follows: dry purge, 10.0 min; trap at maximum temperature, 12.0 min; desorption, 10.0 min; thermostatisation, 45 min. Each cycle took 108.0 min for each chromatographic analysis. The chromatographic separation was carried out in a Clarus 500 gas chromatograph (Perkin Elmer), using a TRB-FFAP column (60 × 0.25 × 0.25 μm), using the following oven temperature program: 3 min at 40 °C, from 40 to 80 °C at 2 °C min^{-1}, 15 min at 80 °C, from 80 to 180 °C at 3 °C min^{-1}, 10 min at 180 °C, from 180 to 210 °C at 5 °C min^{-1}; and 5 min at 210 °C. Carrier gas was He at 3 mL min^{-1}. Detection of different aroma components was carried out by mass spectrometry, using a Clarus 560S mass spectrometer (Perkin Elmer), using EI+ ionisation mode. A mass range of m/z = 25–250 was recorded each 0.5 min, between 9 and 90 min. Ion chromatograms and ion peaks were analysed using Turbomass GC/MS software, v. 5.4.2. (Perkin Elmer) Analyses were carried out in duplicate.

Sensory analysis of wines

Sensory analysis of wines was carried out 1 and 7 months after wines were bottled by ten expert tasters. The tasting sessions were carried out in the 'Escuela Universitaria de Ingeniería Técnica Agrícola' tasting room, using separate tasting booths after ISO 8589 standard (ISO, 1988), using a 9-point scale for the quantification of descriptors (0 = absence, 9 = very high intensity), according to ISO 4121 standard (ISO, 2003). Four preliminary sessions in four consecutive days, using twenty different white wines from *Appellation d'Origine Contrôlé* Rueda and three of the wines under study, were held to allow the generation of olfactory and gustative descriptive terms by the tasters. For this purpose, the A.C. Noble's aroma wheel (Noble *et al.*, 1987) and a series of descriptors for Verdejo wine aroma, based on the literature (Cacho, 2006; Campo *et al.*, 2008) and in the information obtained from experts on wines of *Appellation d'Origine Contrôlé* Rueda, were used. A fifth session, held 3 days after the fourth preliminary session, allowed the generation of the definitive list of descriptors, taking into account the recommendations of ISO 11035 standard (ISO, 1994). Four descriptors were selected for the olfactory phase (tropical fruit, herbaceous, spicy and oak) and three for the gustative phase (acidity, bitterness and softness).

Once descriptors were selected, standard references were prepared for the different descriptors (Table 1), based on literature data (Jackson, 2009); the intensity of references corresponding to five in a 9-point scale. References were prepared, when possible, using analytical degree reagents, which were dissolved in a standard Verdejo wine from Vinos Blancos de Castilla S.A. winery. The standard references were presented to the tasters in separate tasting booths, under controlled temperature and relative humidity.

In June 2008, three tasting sessions were carried out during a week with wines bottle-aged for a month (wines A-1, AB-1 and B-1). Wines were placed in a fridge at 10 ± 1 °C for a day before tasting. Punctuations obtained in these sessions allowed us to obtain the mean value for each descriptor. In January 2009, after two new preliminary sessions, three tasting sessions were

Table 1 Samples used for sensory analysis, based on Jackson (2009)

Sample	Amount for 300 mL of base wine
Tropical fruit	10 mg isoamyl acetate
Herbaceous	3 mg 1-hexen-3-ol
Spicy	60 μg eugenol
Oak	2 g oak wood chips, mid-toast, macerated with wine for 1 month
Acidity	0.7 g tartaric acid
Bitterness	5 mg quinine sulphate
Softness	200 mg Mannoplus®

carried out to evaluate the wines bottle-aged for 7 months (wine A-7, AB-7 and B-7). Samples were evaluated by the same tasters who participated in 2008 sampling. Punctuations obtained in these sessions allowed us to obtain the mean value for each descriptor.

Statistical analysis

The analysis of the results was carried using multifactor ANOVA and *F*-tests techniques (Box *et al.*, 1993), using the Statgraphics 5.0 Plus statistical package (Statistical Graphics Corp., Warrenton, VA, USA). Differences were considered significant at $P < 0.10$.

Results and discussion

General analysis of wines

Table 2 shows that, 1 month after bottling, the most relevant differences for the three wines from the analytical point of view were owing to the higher content of tartaric acid and acidity, both total and volatile. These differences were found in the wines that remained in oak barrels after the alcoholic fermentation (wines AB-1 and B-1). This may be due to three main reasons: the action of the mannoproteins present in the lees, which restrict the precipitation of bitartrates (Lubbers *et al.*, 1993); the partial oxidation of ethanol by *Acetobacter* during wine ageing in oak casks; and the contribution of several acids found in oak wood (Chatonnet *et al.*, 1992).

Table 2 Results of general analysis of wines 1 month after bottling

	Wines		
Analytical parameter	A-1	AB-1	B-1
Alcoholic degree (% vol)	13.25	13.15	13.20
Reducing sugars (g L^{-1})	0.10	0.30	0.15
Total acidity (g L^{-1})*	6.8	7.2	7.5
Volatile acidity (g L^{-1})†	0.28	0.37	0.43
pH	3.72	3.66	3.59
Free SO_2 (mg L^{-1})	14.3	10.6	10.2
Total SO_2 (mg L^{-1})	40.5	41.3	42.4
Tartaric acid (g L^{-1})	3.50	3.60	3.80
Malic acid (g L^{-1})	3.30	3.20	3.30
Citric acid (mg L^{-1})	260	265	255

*Tartaric acid equivalents.
†Acetic acid equivalents.

Wine volatile components

Thirty-five volatile components were identified and quantified in wines. Most of them were esters and alcohols, while fatty acids, lactones, phenols and aldehydes had lower quantities. To know the specific contribution of each volatile component to the aroma of the wine, the aromatic impact values index or olfactive active value (OAV) was used (Falqué *et al.*, 2001). The selected values for the odour detection threshold were the ones proposed by several authors (Chatonnet *et al.*, 1990; Etiévant, 1991; Ferreira *et al.*, 2000; Escudero *et al.*, 2004; Gómez-Míguez *et al.*, 2007).

Four terpenols were identified in the studied Verdejo wines (Table 3). Two of them, linalool and limonene, were present in all the wines, the first one being slightly more abundant, which is in agreement with the results obtained by Herraiz (1988). Nerol and β-citronelol were only detected in wines made totally or partially in stainless steel tanks 1 month after bottling; linalool was slightly more abundant in wine A-1 than in the others. However, limonene presented higher concentrations in the wines made partially or totally in oak barrels (wines AB and B). Although none of the terpenols showed significant differences from the individual point of view for the methodology of winemaking, the sum of those present in all wines (limonene and linalool) was significantly different ($P < 0.10$) for this factor, with an average value of 35 μg L^{-1} for the wines completely fermented in oak barrels, 30 μg L^{-1} for the wines partially fermented in oak barrels and 23 μg L^{-1} for the wine fully fermented in steel tanks. During the ageing in bottles, the concentrations of most terpenols decreased, whether or not the wines were in contact with the lees; two of them (nerol and β-citronelol) completely disappeared. However, the differences were not statistically significant in any of the cases. Two of the studied terpenols exceeded the detection threshold in some of the wines (Table 5): 1 month after bottling, limonene in the wines made with lees in oak barrels (wines AB-1 y B-1) and linalool, also 1 month after bottling, in the wines which were not in contact with the lees (wine A-1).

Two aromatic alcohols were detected and quantified (benzyl alcohol and 2-phenyl ethanol). These components are usually found in small quantities in grapes (Günata *et al.*, 1986) and in the must of Verdejo (Herraiz, 1988); but they are mainly generated during the alcoholic fermentation. While the concentration of benzyl alcohol decreased when the wine was stored in bottles, although not significantly, concentration of 2-phenyl ethanol, being higher, increased when wines were

Table 3 Content of different volatile compounds in wines ($\mu g\ L^{-1}$)

Family of compounds	Volatile compound	Wines					
		A-1	A-7	AB-1	AB-7	B-1	B-7
Terpenols	β-Citronelol	15	0.0	10	0.0	0.0	0.0
	Limonene	4	6	17	8	27	9
	Linalool	27	9	22	12	20	14
	Nerol	14	0.0	0.0	0.0	0.0	0.0
Aromatic alcohols	Benzyl alcohol	740	538	620	597	950	770
	2-Phenylethanol	10 290	6013	6370	7544	5550	6300
Mid-chain alcohols	1-Pentanol	69	41	82	78	90	85
	1-Hexanol	1080	853	960	866	1015	837
	cis-2-Hexenol	21	16	11	16	13	18
	trans-2-Hexenol	13	7	5	9	7	12
	trans-3-Hexenol	180	114	150	144	170	138
	1-Heptanol	710	1098	540	1064	690	587
	1-Octanol	26	19	14	11	14	12
Ethyl esters of fatty acids	Ethyl isovalerate	5.5	5.7	5.7	6.6	6.2	7.7
	Ethyl hexanoate	1260	741	1080	635	900	555
	Ethyl heptanoate	3.2	2.7	3.1	2.5	2.7	2.2
	Ethyl octanoate	2580	1257	2006	932	1480	774
	Ethyl pelargonate	2.7	1.2	1.9	0.9	1.1	0.8
	Ethyl decanoate	900	689	710	499	510	417
	Ethyl dodecanoate	19	18	22	14	45	20
Esters of mid-chain alcohols	Isoamyl acetate	3920	1287	3380	1196	2370	862
	Isoamyl lactate	200	318	214	367	260	492
	Hexyl acetate	61	8	67	3	51	2
	2-Phenylethyl acetate	240	145	160	163	150	292
Ethyl esters of short-chain organic acids	Ethyl piruvate	313	89	157	53	121	41
	Diethyl succinate	4340	5525	4530	8964	5890	8496
	Ethyl lactate	25 320	38 038	25 720	62 615	40 650	74 227
Volatile fatty acids	Butyric acid	480	860	330	1420	400	1150
	Isovaleric acid	2010	1163	3110	7410	1340	3837
	Heptanoic acid	0.0	0.0	0.0	144	0.0	427
	Octanoic acid	19 050	21 852	11 040	21 136	11 280	14 489
Other volatile compounds	Benzaldehyde	80	55	51	46	64	55
	cis-β-Methyl-γ-octalactone	0.0	0.0	360	0.0	220	0.0
	4-Ethylphenol	270	0.0	270	0.0	250	0.0
	Eugenol	4	0.0	45	0.0	260	0.0

made in contact with the lees (wines AB-7 and B-7). This is in agreement with the results obtained by Chung (1986), even though this increase is not significant. Despite the concentration of 2-phenyl ethanol was higher than 5 mg L^{-1} in all the cases, only presented OAV > 1 in wine A-1 (Table 5).

The concentrations of seven mid-chain alcohols identified in the wines can be seen in Table 3. The most abundant ones were 1-hexanol, originated by degradation of grape unsaturated fatty acids by lipooxigenases and which is partly responsible for the herbaceous flavour of young white wines and 1-heptanol. The content of 1-hexanol decreased significantly ($P < 0.10$) as the wine was stored in bottles (Table 4). However, 1-heptanol increased under the same circumstances, unless the wines were completely made in oak barrels; yet, no significant differences were found. 1-pentanol, which mainly generates in the secondary processes of amino acid metabolism, had higher levels in the wines made partially or totally in oak barrels and decreased when the wines were aged in bottles; yet, the differences were not statistically significant. 1-octanol, although it was found in low concentrations, had a lower amount in wines that had been in contact with lees ($P < 0.05$) and still decreased while the wine was kept in bottles. Finally, the three detected hexenols, which are also responsible for the herbaceous flavour of young white wines, had lower concentrations, with no statistically significant differences for any of the studied factors. None of the mid-chain studied alcohols showed concentrations higher than its detection threshold.

Seven fatty acid ethyl esters were identified in Verdejo wines (Table 3). Broadly speaking, these molecules were less abundant in wines that were in contact with the lees

Table 4 Results of ANOVA for several chemical parameters

	Winemaking technology		Length of conservation in bottle	
Chemical parameter	***F***	***P***	***F***	***P***
Sum of limonene and linalool	9.75	0.09	69.11	0.01
1-Hexanol	0.67	0.60	18.12	0.05
1-Octanol	23.01	0.04	8.05	0.10
Ethyl hexanoate	9.79	0.09	74.99	0.01
Ethyl heptanoate	79.00	0.01	256.00	0.00
Ethyl octanoate	6.53	0.13	33.27	0.03
Ethyl decanoate	23.82	0.04	19.13	0.05
Isoamyl acetate	3.17	0.24	41.53	0.02
Isoamyl lactate	4.62	0.18	26.92	0.04
Hexyl acetate	1.45	0.41	146.02	0.01
Ethyl pyruvate	2.72	0.27	9.36	0.09
Diethyl succinate	2.16	0.32	8.50	0.10
Ethyl lactate	3.86	0.21	13.43	0.07

(wines AB and B); yet, only ethyl hexanoate, ethyl heptanoate and ethyl decanoate had statistically significant differences in this case (Table 4). Thus, the presence of a larger quantity of lees resulted in a minor concentration of these esters, bearing in mind the obtained mean values for each type of elaboration. These data may be due to several reasons: firstly, because the ethylic esters of fatty acids may settle in the insoluble fraction that appears in the autolysis of yeasts, in agreement with several authors (Voilley *et al.*, 1990; Comuzzo *et al.*, 2011); secondly, owing to adsorption in oak wood (Ramirez-Ramirez *et al.*, 2004; Comuzzo *et al.*, 2011); and finally, as a result of the hydrolysis of esters produced by enzymes released by yeasts (Mauricio *et al.*, 1993). The ageing in bottles results in the decreasing content of six fatty acid ethyl esters, as previously pointed out (Flanzy, 2003). This decrease was statistically significant ($P < 0.05$) for ethyl hexanoate, ethyl heptanoate, ethyl octanoate and ethyl decanoate (Table 4). The results of the statistical analysis seem to suggest the existence of an important influence of the interaction between time kept in the bottle and the method of winemaking. Yet, the analysis of variance seems to be inadequate to detect that in a more accurate way, owing to the fact that the reduced number of available samples does not allow considering the effect of each factor and justifying, in a better way, the detected variations. Three of the studied esters (ethyl isovalerate, ethyl hexanoate and ethyl octanoate) showed an OAV > 1 for all the wines (Table 5).

Four volatile components were identified as esters of mid-chain alcohols and three different ones as ethyl esters of short-chain organic acids (Table 3). Two of them, (isoamyl acetate and hexyl acetate) degraded quickly ($P < 0.05$) during the storing in bottle (Table 4). The levels of 2-phenylethyl acetate from the wines that had been in contact with the lees after the alcoholic fermentation increased with the ageing of wine in bottle, which is in agreement with the previous reports (Chung, 1986; Herjavec & Majdak, 2002). However, the differences are not statistically significant. This increase was parallel to that of 2-phenylethanol (Table 3) in wines aged on lees. The most abundant of them was ethyl lactate (Table 3). The concentrations of ethyl lactate, isoamyl lactate and diethyl succinate increased when the wines were kept in bottles for 7 months; this may be a consequence of the development of esterification reactions; these reactions seemed to be more intense for lactates. These results are in agreement with previous reports showing that the esterification of the monocarboxylic acids is faster than that of dicarboxylic acids

Table 5 Of olfactive active value for different aroma compounds in wines

		Wines					
Aroma compound	**Perception threshold (μg L^{-1})**	**A-1**	**A-7**	**AB-1**	**AB-7**	**B-1**	**B-7**
Limonene	15*	0.24	0.40	1.13	0.54	1.80	0.61
Linalool	25[†]	1.08	0.37	0.88	0.48	0.80	0.58
2-Phenyletanol	10 000[‡]	1.02	0.60	0.63	0.75	0.55	0.62
Isovaleric acid	2000[§]	1.00	0.58	1.55	3.70	0.67	1.91
Octanoic acid	500[‡]	38.10	43.70	22.08	42.27	22.56	28.97
Ethyl isovalerate	3[‡]	1.83	1.90	1.90	2.20	2.06	2.56
2-Phenylethyl acetate	250[§]	0.96	0.57	0.64	0.65	0.60	1.16
Ethyl hexanoate	14[‡]	90.00	52.90	77.14	45.36	64.28	39.62
Ethyl octanoate	5[‡]	516.0	251.40	401.20	184.66	296.00	154.86
Isoamyl acetate	30[‡]	130.66	42.89	112.66	39.85	79.00	28.74
Eugenol	7[¶]	0.57	–	6.42	–	37.14	–
cis-β-Methyl-γ-octalactone	46[§]	–	–	7.84	–	4.78	–

*Etiévant, 1991; [†]Ferreira *et al.*, 2000; [‡]Gómez-Míguez *et al.*, 2007; [§]Chatonnet *et al.*, 1990; [¶]Escudero *et al.*, 2004.

(Shinohara & Watanabe, 1978; Herjavec & Majdak, 2002). The highest levels of these esters were found in the wines aged on lees (wines AB and B), which may be due to the fact that the release of lactic and succinic acids by the lees help esterification (Chung, 1986). The analysis of variance (Table 4) showed that the time the wine was stored in bottles had a significant influence on the levels of hexyl acetate, isoamyl acetate, isoamyl lactate ($P < 0.05$) and also ethyl piruvate, diethyl succinate and ethyl lactate ($P < 0.10$). Only two acetates had a value of OAV > 1 in some samples (Table 5): isoamyl acetate in all the wines and 2-phenylethyl acetate only in wine B-7: this was completely made in oak barrels and stored in bottles for 7 months. Isoamyl acetate, with an OAV > 1 in all the wines (Table 5), is the ester that most affects the aroma, although its incidence on the olfactory stage was higher when the wine was only made in stainless steel tanks. That incidence diminished when the time that wine was stored in bottles was longer. As mentioned earlier, several esters had an OAV > 1, being the values of OAV very high in three cases (ethyl hexanoate, ethyl octanoate and isoamyl acetate). These compounds provide wines with olfactory features related to fruits. As the OAV values of these esters depended on the methodology of elaboration and the time of storing in the bottles (Table 5), the fruity features of the Verdejo wines were highly determined by these factors, as it is explained later on.

Four volatile fatty acids were detected (butyric, isovaleric, heptanoic and octanoic acids), being octanoic acid the most abundant one (Table 3). This acid exceeded the detection threshold in all the cases, so that OAV > 1 in all the samples (Table 5). If the wines were made only in stainless steel tanks, its levels were higher than in those wines made partially or completely in oak barrels. This is in agreement with other results on the decreasing amount of mid-chain fatty acids (C-6–C-11) when wines were aged on lees (Lafont-Lafourcade *et al.*, 1984; Shinohara, 1985). The content of octanoic acid increased in all the cases when wines were stored in bottles unlike ethyl octanoate, because this ester is hydrolysed during storing in bottles (Table 3). The content of the three other fatty acids usually increased during the storing of wines in bottles. Of the three, the isovaleric acid was the most abundant one, so that OAV > 1 (Table 5).

Four more volatile components were identified: one lactone, one aldehyde and two volatile phenols (Table 3). Only the wines made in oak barrels and stored in oak barrels for a month had measurable quantities of *cis*-β-methyl-γ-octalactone, which is found to be above its perception threshold, so that OAV > 1 (Table 5). This lactone was not present in the rest of the wines, and *trans*-β-methyl-γ-octalactone was not detected in any of them. These lactones appear in the wines as a result of being in contact with the wood of the oak barrels, but according to the obtained results they disappeared while the wine was stored in bottles, which was also perceived in the sensory analysis (see section 3.3). Benzaldehyde, with a typical aroma of sour almond, showed low levels 1 month after bottling: these levels decreased with the ageing of wine in bottles (Table 3).

Although other authors have reported on the potential of the yeast walls to absorb volatile phenols, especially 4-ethylphenol (Chassagne *et al.*, 2005), the concentrations of 4-ethylphenol 1 month after ageing in bottles were very similar, ranging from 270 to 250 μg L^{-1}, although slightly lower in the wines made completely in oak barrels (Table 3). During the storing in bottles, it disappeared, with the result that 7 months after bottling it could not be quantified. One month after bottling, significant quantities of eugenol were found, which conveys smoky and spicy features (Table 3). Its content was higher in the wines made partially or totally in oak barrels. (AB-1 and B-1), exceeding the detection threshold, so that OAV > 1 (Table 5). Generally, it is thought that it generates when the wine is in contact with oak barrels, but its presence in wines made exclusively in stainless steel tanks suggests that it may be generated in different ways. This phenol, like 4-ethylphenol, disappeared when the wine was stored in bottles for 7 months.

Sensory analysis of wines

The results of the sensorial analysis are shown in Table 6. It can be observed that the two studied factors have a significant influence on several of the analysed descriptors.

In the olfactory phase, 1 month after bottling, the wine made completely in stainless steel tanks (A-1 wine) had a stronger aromatic intensity, especially that of tropical fruit. However, the oak and spicy aromas ($P < 0.10$) were predominant in the wines made partially or completely in oak barrels. Throughout the storing of wines in bottles, the assessment of the olfactory descriptors decreased in all the cases, the losses of tropical fruity aromas being significant ($P < 0.05$); these are related to a lower concentration of esters (Table 3) and of variety thiols (Campo *et al.*, 2005). In addition, it was also perceived a lower perception of spicy aroma ($P < 0.10$) in these wines. These results are in agreement with those mentioned by other authors (Flanzy, 2003; Jackson, 2009).

In the tasting phase, the acid and bitter sensations decreased while the wines were stored in bottles, the loss of bitterness being significant ($P < 0.10$). In all the cases, the acidity was more intense in the wines made partially or totally in oak barrels; to some extent, this is owing to the contribution of wood that provides acid components. However, the sensation of bitterness in the wines made exclusively in stainless steel tanks was more intense ($P < 0.10$), probably due to a variety characteristic and to the use of skins maceration. The wines made partially or totally in oak barrels have a higher

Table 6 Values for different attributes determined in sensory analysis of wines. Each attribute was scored between 0 and 9

	Wines						Winemaking technique		Length of conservation in bottle	
Attribute	**A-1**	**A-7**	**AB-1**	**AB-7**	**B-1**	**B-7**	***F***	***P***	***F***	***P***
Tropical fruit	6.8	4.1	5.6	3.8	4.9	3.4	4.37	0.18	30.77	0.03
Spicy	0.6	0.2	3.2	1.2	3.5	1.8	7.88	0.11	7.75	0.10
Herbaceous	2.9	1.7	2.2	2.0	2.4	1.9	0.16	0.85	4.57	0.16
Oak	0.3	0.8	5.5	3.8	4.7	3.1	12.35	0.07	1.69	0.32
Acidity	5.4	5.5	6.1	5.8	5.8	5.4	3.76	0.21	1.71	0.32
Bitterness	4.6	4.5	4.3	3.9	4.0	3.5	14.85	0.06	7.69	0.10
Softness	4.1	4	4.6	4.9	5.4	5.6	48.54	0.02	1.23	0.38

content of mannoproteins, as these are released by the lees and convey a slight sweet flavour that masks bitterness (Vidal *et al.*, 2004a). The making of wine in oak barrels, either totally or partially, and thus the ageing on lees, resulted in wines with higher softness ($P < 0.05$). This sensation increased in these wines as lees were in contact with wine for a longer period during winemaking, because mannoproteins contribute to that sensation (Vidal *et al.*, 2004b) and also when the time of storing was increased.

Conclusions

The use of oak barrels during the alcoholic fermentation, both partial and total, as well as during the ageing, which means the wines were in contact with lees, changed the aroma composition and the sensory characteristics of the wines. As a result, the levels of several volatile components (1-octanol, ethyl heptanoate and ethyl decanoate) and the assessment of several descriptors used during the sensory analysis (oak, bitterness and softness) changed significantly. On the other hand, the time the wines were kept in the bottles significantly changed the levels of several esters, 1-hexanol and 1-octanol, and also of three sensory attributes (tropical fruit, spicy and bitterness). The study of OAV showed that twelve of the evaluated components had a higher value than the unit in some of the wines, and five of them in all the wines, most of them being esters. This fact was reflected by the high scores obtained by the attribute 'tropical fruit' in sensory analysis. For all these reasons, the composition and the quality of the aroma of Verdejo white wines appear to be significantly affected both by use of oak barrels in winemaking and by the time the wines are stored in bottles.

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